Report and Recommendation from the RAS Ad Hoc Working Group on the NCI RAS Initiative

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The NCI RAS Initiative

- First National Missions Program, sponsored by NCI in 2013
- Overarching goal to mobilize the cancer research community to develop ways to understand and target cancers driven by mutant KRAS in an open research model of collaborations among government, academic, and industrial researchers
- State-of-the-art facilities at the Frederick National Laboratory for Cancer Research (FNLCR) serves as a hub for the RAS Initiative and collaborating laboratories function as spokes
- Initial funding cycle addressed critical knowledge gaps that had impeded or discouraged pursuit of RAS as a drug target
- Program was reviewed and renewed in 2017 with a recommended increased focus on i) identifying and advancing RAS therapeutic compounds and ii) developing a biophysical model of RAS activation and signaling in the membrane
- The NCI RAS Initiative's Request for Renewal will take place in 1Q FY 2023

Mission of the FNLAC RAS Ad Hoc Working Group

- To advise strategic, technical, and scientific aspects of the NCI RAS Initiative
- To present findings and recommendations to the Frederick National Laboratory Advisory Committee (FNLAC) and other relevant working groups
- Overall WG goals:

(1) Provision of highest quality oversight to Drs. F. McCormick & D. Nissley and the FNLCR team

(2) Regular and candid assessments of progress and suggestions for improvements or pivots

(3) Ensure optimal connectivity between the FNLCR RAS initiative and the extramural community

- Nine WG meetings held between Sept. 2018 and Aug. 2022
- Today's objective
  - Review progress during current program cycle (FY18-22)
  - Provide recommendations and overall enthusiasm for the NCI RAS Initiative

RAS Dependencies and Membrane Biology

- Developed cell culture systems to identify distinguishing properties of RAS mutants
  - Hyperactive glutamine and redox metabolism facilitate chemotherapeutic resistance in KRAS mutant pancreas cancer cells (Cancer Res)
  - Comprehensive update of the frequency of RAS isoforms and alleles that drive cancer (Cancer Res)
  - Heightened intrinsic KRAS-G13D GTP exchange activity is susceptible to extrinsic GAPs, pointing to GTP exchange inhibition as a potential therapeutic target in G13D neoplastic cells
- Top-down mass spectroscopy methods have enabled unprecedented analyses of intact KRAS-4B “proteoforms” in cancer cells, revealing distinct post-translational modifications on KRAS oncoprotein subpopulations
- Developed synthetic membrane systems to test hypotheses about KRAS signaling
  - KRAS diffusion is governed by the hypervariable region/lipid interactions until larger order complexes are formed (Elife)

Targeting RAS and RAS-Effector Interactions

- Crystal structures of wild-type and mutant KRAS, +/- tool compounds and leads
  - Structural understanding of covalent inhibitors has translated “hits” into “lead” RAS inhibitors
- First structure of RAF1 RAS-binding domain (RBD) and its cysteine-rich domain (CRD) bound to wild-type and mutant RAS (Nature Comm)
  - Insight to RAF1 activation and new therapeutic opportunities
- First structure of KRAS/PI3Ka (p110α/p85)
  - Developed “complex breaker” with improved safety profile
  - 35 RAS/PI3Kα co-structures solved
- First to demonstrate Neurofibromin 1 (NF-1) forms high-affinity dimers (JBC, Cell Rep)
  - Discovered ternary complex NF1, SPRED-1 (EVH1 domain), and active KRAS; new target efforts launched

RAS Biophysics, Molecular Dynamics, and Computational Biology

- Demonstrated a major subpopulation of KRAS conformers is displaced from the membrane and available to promote RAF activation
    - Model predicts that RAS conformers may be regulated by post-translational modification, protein-protein, and protein-lipid interactions (PNAS)
- Molecular dynamics and surface plasmon resonance to measure and predict the behavior of RAS-RAF complex with lipid bilayer constituents
    - Suggests important role for anionic lipids in stabilizing RAS-RAF complexes at the membrane (JBC)
- Multi-year collaboration with LLNL used matched supercomputing and machine learning to develop a multiscale computational model that allows simulations of 100s of RAS and RAF molecules on a realistic (complex, 8 lipids) membrane at time scales that enable statistically relevant observations and generate hypotheses (PNAS)

RAS Drug Discovery and Development

- Lead G12C RAS inhibitor development was accelerated via strategic partnership
  - Next-generation inhibitor exhibits improved kinetics and potency, binds RAS in active (GTP) and inactive (GDP) states, and is active *in vivo*; anticipated to enter clinical development in 2023
- First-in-class RAS/PI3Ka complex small molecule disruptor with improved safety profile
  - Distinct small molecule series identified with strategic partner, diminished risk for hyperglycemia, anticipated to enter clinical development in 2023
- Multiple discovery-level projects directly targeting KRAS or RAS effector and regulators
  - Direct acting, covalent and non-covalent binding inhibitors for other mutagenic RAS alleles
  - KRAS proteolysis-targeting chimeras (PROTACs)
  - RAS effector/regulator interactions via active screening and *in silico*/molecular modeling approaches

Fostering Interactions with the Scientific Community

- Promote and mediate the sharing of knowledge and resources among RAS researchers in the academic, government, and private sectors
- >66 publications in peer-reviewed, high impact journals
  - Contributed to RAS and RAS effector “druggability” concept, helped de-risk the field
- Major RAS reagent hub/distributor of protocols, plasmids, vectors, proteins, cell lines
  - >12,000 plasmids, 63 proteins, >1,000 cell lines to >1,300 investigators (since 2018)
- RAS Symposia Sponsor; promote discourse, information and breakthrough
  - Two symposia: FY 2021 (virtual) and FY 2023 (in person, Oct 2022)
- Scientific communication support via The NCI RAS Initiative (cancer.gov/RAS) and RAS Lab Discussion form
  - Website being refreshed to streamline format, speak to lay community, and update the science

Conclusions

- The NCI RAS Initiative has exceeded its objectives since its inception, despite COVID-19 challenges
- Two clinical candidates are headed towards IND and clinical evaluation (2023)
  - KRAS G12C covalent binding inhibitor with differentiating characteristics
  - KRAS/PI3Kα “complex breaker”, a first-in-class approach
- Exceptional capabilities in structural, biochemical, and biophysical approaches have enabled targeting of the more common KRAS-G12D and KRAS-G12V oncoproteins and broadened the target landscape to include RAS effector and regulator proteins
- Impactful studies related to RAS biochemistry and regulation; NF1 can form homodimers and explains why certain NF1 alleles function as dominant negatives
- Biophysical and modeling revealing RAS exists in dynamic states on the plasma membrane, with implications in signaling and drug development

Future Directions and Recommendations

- Continue efforts to translate homegrown NCI RAS Initiative compounds to the clinic
  - The KRAS G12C inhibitor has unique properties; this compound and other KRAS-G12D and pan-KRAS compounds advancing toward clinical translation should continue to be prioritized.
  - Understanding and discovery of mechanisms to combat therapeutic resistance. Therapeutic resistance will remain a challenge in treating RAS-mutant tumor types.
- Continue the pursuit of secondary targets such as the PI3Kα-RAS “complex breaker” compound for use as salvage or combination therapy
- Continue to catalyze the renaissance in RAS targeting approaches on a global scale, including the dynamics of RAS activation, and the exploration of inhibitors for additional RAS family members
  - Discoveries related to movement and dynamics of RAS at the membrane are re-shaping how the Research Team thinks about therapeutic development
  - Exploration of inhibitors for additional RAS family members altered in cancer is timely and important.
  - Pursue additional targets only if additional resources can be provided to allow for expansion without taking away from successful ongoing efforts to target KRAS-mutant disease
- Expansion of community engagement to further the impact of the NCI RAS Initiative